P-1347

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61L 15/36, A61F 13/15

A1

(11) International Publication Number: **WO 99/17813**

(43) International Publication Date: 15 April 1999 (15.04.99)

(21) International Application Number: PCT/SE98/01816

(22) International Filing Date: 8 October 1998 (08.10.98)

(30) Priority Data:
9703669-3 8 October 1997 (08.10.97) SE

(71) Applicant *(for all designated States except US)*: SCA HYGIENE PRODUCTS AB [SE/SE]; S-405 03 Göteborg (SE).

(72) Inventors; and

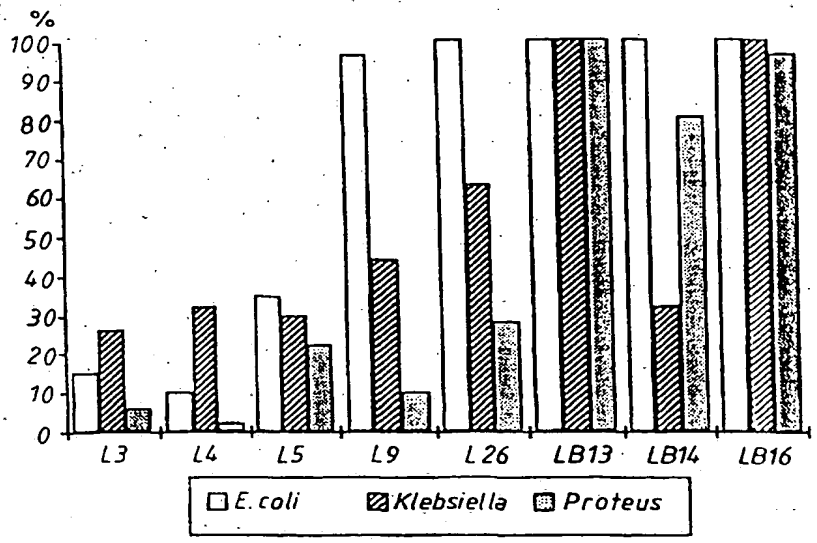
(75) Inventors/Applicants *(for US only)*: ANDERSSON, Rolf [SE/SE]; Vallmovägen 11, S-435 31 Mölnlycke (SE). RUNEMAN, Bo [SE/SE]; Jons Väg 6, S-433 75 Jonsered (SE). FORSGREN-BRUSK, Ulla [SE/SE]; Plommonvägen 35, S-435 43 Mölnlycke (SE). GRAHN HÅKANSSON, Eva [SE/SE]; Nygatan 74, S-903 31 Umeå (SE). HÅKANSSON, Stellan [SE/SE]; Nygatan 74, S-903 31 Umeå (SE). HOLM, Stig, E. [SE/SE]; Norra Gimonäsvägen 25, S-907 38 Umeå (SE).

(74) Agents: BERG, S., A. et al.; Albihns Patentbyrå Stockholm AB, P.O. Box 3137, S-103 62 Stockholm (SE).

(81) Designated States: AU, BR, CA, CN, CZ, EE, HU, ID, IL, JP, KR, MX, NO, NZ, PL, RU, SK, TR, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: PRODUCT AND PREPARATION CONTAINING LACTIC ACID BACTERIA



(57) Abstract

Absorbent articles comprising lactic acid bacteria, and which articles can be stored for a long time under non-ideal conditions can be obtained by applying a suspension of lactic acid bacteria to an absorbent product, whereafter the absorbent product is dried to a moisture content of less than 10 %, preferably less than 5 %, and most preferably less that 1 %, calculated as percentage of weight of the absorbent core in the product. The absorbent product can be a diaper, sanitary napkin, panty liner, incontinence guard or like article. As already mentioned, it contains lactic acid bacteria and the article is intended to be carried in contact with the user's skin in the perineum area, wherein lactic acid bacteria are arranged to be transferred to the user's skin and, when applicable, to the mucus membrane in the perineum area, to form a microbiological barrier that impairs the conditions for spreading and establishment of undesirable stains of microorganisms in said perineum area.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

Groups that are even more at risk in this respect are young girls who do not yet have a developed flora of lactic acid bacteria in the urogenital region, and older women who no longer have a developed flora of this nature.

Another risk group are individuals who have been treated with antibiotics against some other type of infection, resulting in a change in their general, natural microbiological flora and then also in the urogenital region.

A closely related problem resides in vaginal colonisation from the anus of, e.g., streptococci, etc. A large percentage of adult women (~30%) carry group B streptococci vaginally. Pregnant women from this group are a particular risk group, since the foetus or the newly born child can be contaminated and develop a serious infection.

A closely related problem is bacterial vaginosis. A large proportion of adult women (~10%) suffer from this. Pregnant women that have such problems constitute a risk group, since the condition can lead to a premature birth which constitutes a serious risk to the child's health.

Recurrent urinary tract infections are general in the case of many individuals, and the occurrence of such infections can lead to complications in the form of kidney damage, for instance, when relevant treatment is not available.

A natural part of the prophylaxis against infections in the urogenital region is enhanced personal hygiene. However, it can be unsuitable to wash genitals and lower abdomen with an excessively strong soap and bactericidal substances, and consequently it may be difficult for an individual to reduce the risk of infection to a sufficient level with the aid of conventional means.

PRODUCT AND PREPARATION CONTAINING LACTIC ACID BACTERIA

FIELD OF INVENTION

The present invention relates to an absorbent article that contains lactic acid bacteria and that is intended to be brought into contact with a user's skin in the perineum.

BACKGROUND OF THE INVENTION

Infections in the urogenital region is a problem that affects many individuals. In and around the anus, there are many different kinds of microorganisms as well as a large amount of these microorganisms. It is known that one reason for many infections in the urogenital region is that microorganisms from a person's own intestinal flora spread from the anus to urogenital organs over perineum and there cause infection.

Normally, an ecological balance prevails between different microorganisms on skin and mucus membrane, and the normal microbiological flora is highly significant in preventing the establishment of undesirable microorganisms. Lactic acid bacteria, *inter alia*, play an active role in this respect. However, situations are found where this natural defence system is inadequate and is disturbed in a way that enables potentially pathogenic microorganisms to become established and give rise to infection, for instance in conjunction with medication, poor hygiene, skin changes and changes in mucus membranes.

Microorganisms that can be associated with the occurrence of these problems are, e.g., microorganisms from the genera Escherichia, Enterococcus, Proteus, Klebsiella, Streptococcus, Gardnerella and Candida.

With regard to the danger of contracting infections in the urogenital region, old and young women are more at risk than men, due to the short distance from the anus to the urethra orifice and vagina.

Traditionally, the aforesaid problems are addressed by treating an infection with conventional antibiotics. However, frequent treatment with antibiotics leads to the development of resistant bacteria strains, which can make continued treatment of new infections very difficult. A further problem with antibiotic treatment is that many individuals are hypersensitive to antibiotics. Yet another problem with antibiotic treatment is that the microbiological flora in and around the anus is complex and relatively undefined. It is therefore difficult to propose a prophylactic treatment for reducing the risk of the occurrence of infections that can be caused by microorganisms from the intestines.

Hitherto, the only available method for reducing the risk of infection in the urogenital region has been treatment with antibiotics. However, since the use of antibiotics for a prophylactic purpose is unsuitable for several reasons, there is a serious need for an alternative solution to the problem by generating and maintaining a desired microbiological flora in the urogenital region.

DESCRIPTION OF THE BACKGROUND ART

As earlier mentioned, a traditional method of dealing with the aforesaid problems is to treat the patient with conventional antibiotics. Various alternative methods of dealing with the aforedescribed problems have been proposed. The use of bacteria as so-called probiotics as an alternative to antibiotics is part of this new methodology.

It is known that certain lactic acid bacteria can have an inhibiting effect on other microorganisms. Application of such lactic acid bacteria has been found to prevent the occurrence of infections on both skin and mucus membrane.

Medical use of selected strains of lactobacteria is described in Canadian Patent Specification CA 1298556 (Bruce, Reid), where, among other things, whole cells or fragments of cells of Lactobacillus are used to treat or to prevent the occurrence of urinal tract infections and intestinal infections.

International Patent Application WO 93/09793 (Reid) describes the use of lactobacteria and skim milk preparations to prevent the occurrence of urogenital infections.

Both CA 1298556 (Bruce, Reid) and WO 93/09793 (Reid) describe the ability of the microorganisms to fasten to the mucus membrane walls, e.g. to the uroepithelium cells or vaginal epithelium cells as an important component for the function of the treatment. However, neither CA 1298556 (Bruce, Reid) nor WO 93/09793 (Reid) describes how the substance concerned shall be applied to the user.

International Patent Application WO 92/13577 (Kvanta) teaches a tampon or sanitary napkin that has been impregnated with a culture of lactic acid producing bacteria, preferably of the genus Pediococcus, that has been isolated from healthy individuals. The tampon or sanitary napkin is intended for prophylactic treatment of urogenital infections.

WO 92/13577 (Kvanta) teaches that applied microorganisms of Lactobacillus are attenuated when technically handled, and the method described relates to treatment and prophylaxis around the actual urethra orifice.

The above cited documents are all solely directed to administration of lactic acid bacteria to the skin. However, none of the documents discloses anything about providing absorbent articles comprising lactic acid bacteria, which articles can be stored for a long time and still contain a sufficient amount of viable and transferable bacteria. It is absolutely necessary that consumer products such as absorbent articles can be stored for a long time and under non-ideal conditions without risking that the quality of the articles is impaired. Consequently, there is a need for absorbent articles which articles are specially adopted for long-time storage under unfavourable conditions.

SUMMARY OF THE INVENTION

It has now turned out that the above mentioned problems can be overcome, and a high quality can be ensured, by applying a suspension of lactic acid bacteria to an absorbent product, whereafter the absorbent product is dried to a moisture content of less than 10 %, preferably less than 5 %, and most preferably less that 1 %, calculated as percentage of weight of the absorbent core in the product. The absorbent product can be a diaper, sanitary napkin, panty liner, incontinence guard or like article. As already mentioned, it contains lactic acid bacteria and the article is intended to be carried in contact with the user's skin in the perineum area, wherein lactic acid bacteria are arranged to be transferred to the user's skin and, when applicable, to the mucus membrane in the perineum area, to form a microbiological barrier that impairs the conditions for spreading and establishment of undesirable stains of microorganisms in said perineum area.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be described in greater depth with reference to the accompanying drawings.

Fig. 1 shows the results obtained with interference tests between selected lactic acid bacteria and undesirable microorganisms.

Fig. 2 illustrates schematically the construction of an inventive test product in the form of an insert.

DETAILED DESCRIPTION OF THE INVENTION

Thus, an inventive absorbent article is intended to transfer lactic acid bacteria to the wearer's perineum and thereby produce a microbiological flora that impairs in the perineum the living conditions for undesirable strains of microorganisms. Undesirable

microorganisms are therewith prevented from spreading from the wearer's anus to the urogenital organs.

The lactic acid bacteria, or a preparation that contains lactic acid bacteria, is applied to an absorbent article such as a diaper, sanitary napkin, panty liner, incontinence guard and a like article.

Generally, the lactic acid bacteria are applied to the article by adding a suspension of said bacteria to the surface of the article that is intended to face the wearer. Preferably, this suspension contains skim milk. It is advantageous that the suspension is concentrated in order to reduce the total volume of the suspension, but on the other hand a highly concentrated suspension may lead to clogging problems in pipes and nozzles. A suitable concentration is within the range from 1×10^9 to 1×10^{12} cfu/ml (colony-forming units per ml), and preferably within the range from 1×10^{10} to 1×10^{11} cfu/ml. This suspension can be added by spraying or pouring it onto the article. The aqueous part of the suspension is immediately absorbed into absorbing layers in the lower part of the article leaving the bacteria in the upper part of the article that is closest to the wearer in a substantially dry state. Despite this absorption, it has turned out to be essential to carry out a drying step in order to ensure that the moisture content of the finished article is less than 10 %, preferably less than 5 %, and most preferably less than 1 %, calculated as percentage of the weight of the absorbent core in the product.

The lactic acid bacteria will preferably have an inhibiting effect on the growth of undesirable microorganisms in the perineum.

Lactic acid bacteria that are suitable for use in accordance with the invention include one or more strains of the genera Lactobacillus, Lactococcus or Pediococcus. However, the lactic acid bacteria will preferably consist of one or more strains from the Lactobacillus genus.

The lactic acid bacteria can also be combined with other components, such as a pH-lowering substance or an auxiliary substance that will facilitate survival of the lactic acid bacteria. Powdered skimmed milk is one example of such an auxiliary substance.

An inventive absorbent article can be combined advantageously with the local administration of lactic acid bacteria in the vagina with the aid of a capsule, vagitorium or some like means. The inventive substance can also be used advantageously in direct conjunction with antibiotic treatment, for instance the treatment of a urogenital infection.

The invention can be applied with all types of absorbent articles that are intended to be worn in contact with the user's perineum. The lactic acid bacteria may therewith be included as a component in the absorbent body of the absorbent article, or applied in or on the liquid-permeable casing sheet of the article, or in or on a liquid transport layer between the liquid-permeable casing sheet and the absorbent body, or may be applied on a separate carrier, such as a tissue layer or the like. The absorbent article may be a diaper, a sanitary napkin, an incontinence guard, or a panty liner. Such articles normally include an absorbent body or pad enclosed in a casing, wherein the casing suitably includes a liquid-permeable outer sheet over the surface that is intended to lie proximal to the wearer in use. An advantage is afforded when a liquid barrier layer, for instance in the form of plastic film, is arranged adjacent the opposite surface which lies distal from the wearer in use.

When the lactic acid bacteria are disposed inwardly of the article casing, it is essential with respect to the invention that the bacteria are able to pass through the casing at that surface of the article which is intended to lie against the wearer's skin in the perineum.

It has been found to be advantageous when the number of lactic acid bacteria in the absorbent article is between 10^4 cfu and 10^{11} cfu, and preferably between 10^6 cfu and 10^{10} cfu.

Alternatively, the absorbent article may have the form of an insert that includes means for its attachment to the liquid-permeable outer sheet of a conventional absorbent article. One advantage with the use of such an insert is that it avoids the need to produce products provided with lactic acid bacteria in a large number of different sizes and models.

As already mentioned, the object of the present invention is to provide an absorbent article of the aforementioned kind that will reduce spreading of bacteria from the anus to the urogenital organs with the aid of microbiological antagonism. This object has been achieved in accordance with the invention with an absorbent article that includes lactic acid bacteria. The absorbent article is intended to be used regularly and in contact with the wearer's skin in the perineum area. By regular use may, in this case, mean daily use or almost daily use of the inventive article. The lactic acid bacteria are therewith applied to the user's perineum area so as to produce and maintain in this area a microbiological flora that impairs the living conditions for undesirable microorganisms in said perineum area, and therewith prevent spreading of these microorganisms from the anus to the urogenital organs.

Examples of species that are associated with urogenital infections are Escherichia coli, Enterobacter, Klebsiella, Pseudomonas, Proteus, Staphylococcus saprophyticus, Staphylococcus epidermidis, group B streptococci, Enterococci, Candida sp., Clamydia sp., Gardnerella vaginalis, Mobiluncus and Bacteroides sp.

As before mentioned, the invention is based on microbiological antagonism. Microbiological antagonism implies that one microorganism or combinations of microorganisms will inhibit other microorganisms. An antagonistic strain shall exhibit growth inhibiting effects with conventional interference techniques on several of the aforesaid undesirable microorganisms. Other important requirements of a suitable antagonistic microorganism is its ability to survive during storage and its growth ability or ability to maintain its activity in a product during use.

Antagonistic microorganisms may be naturally occurring organisms that are non-toxic and that do not exert any negative biological effect on human beings, in the form of infection or skin changes. However, antagonistic microorganisms can also be produced by biotechnical processes.

Certain strains of lactic acid bacteria have a powerful inhibiting effect on undesirable bacteria strains in the user's perineum. This inhibitory effect has its foundation in the fact that lactic acid bacteria possess a number of antagonistic properties that act through different mechanisms, such as by lowering pH, e.g. by producing lactic acid, competition for available nutrients, reduction of the redox-potential, production of hydrogen peroxide, production of specific inhibiting substances or components, such as enzymes, toxins or bacteriocines and competition for available binding sites. The antagonistic effect can be elevated still further in some instances, by adding an additional pH-lowering substance.

The growth of undesirable microorganisms present in the perineum of the user can be inhibited by adding to the inventive product lactic acid bacteria that exhibit antagonistic properties against said undesirable microorganisms. At least some microorganisms of undesired species can also be killed in this way. The microorganisms added to the product must be added in such quantities and have such activities as to achieve the effect desired. This effect is normally achieved when the number of antagonistic microorganisms per product is greater than 10^6 cfu, preferably 10^8 cfu and more preferably 10^9 cfu.

One advantage afforded by the use of antagonistic microorganisms is that one avoids an undesired selection pressure on the microenvironment, such as favouring of potentially pathogenic microorganisms and therewith the risk of developing pathogenic strains that are resistant to antibiotics and chemopharmaceuticals. Since the antimicrobial system is based on a natural biological process, the risk of ecological and toxic environmental disturbances is reduced.

The inventive product can include a carrier in the form of, e.g., a typical panty liner with or without a liquid-impervious backing sheet and including an absorbent layer which contains 100-200 g/m^2 cellulose pulp mixed with 0-10% superabsorbent powder. That side of the product which is intended to lie proximal to the wearer in use includes lactic acid bacteria in a concentration that will preferably be in the order of 10^4-10^{11}, preferably 10^6-10^{10} cfu per product. An inventive product will preferably include a suitable substance that will assist in the survival of the microorganisms. One such aid may, e.g., be powdered skim milk. Regardless of the general form of the product, an inventive product may also include a suitable pH-lowering substance for further enhancing the antagonistic effect.

EXAMPLES

The following examples are intended to further illustrate the effect of an inventive product.

Example 1

Bacterial antagonism was studied with the aid of tests carried out in accordance with the agar overlay method. The method is based on the diffusion of the growth inhibiting substance produced by the lactic acid bacteria through an agar layer and their inhibition of the growth of the test organisms.

Lactic acid bacteria, three strains of Lactobacillus designated LB13, LB14 and LB16 respectively, and five strains of Lactococcus designated L3, L4, L5, L9 and L26 respectively, were cultivated to an overnight culture in nutrient broth from Merck. Lactococcus were cultivated in M17 and Lactobacillus in MRS. Agar (2%) of M17 and MRS (25 ml) respectively was mixed with 1.0 ml of respective bacteria and moulded in a Petri dish. The agar plates were incubated overnight at 37°C. The plates with MRS were incubated in a CO_2 atmosphere. Reference plates were prepared in a corresponding

manner, but without lactic acid bacteria. A fresh layer containing 25 ml agar was cast on top of the existing layer in the Petri dishes and allowed to solidify.

The test organisms in the form of gram-negative bacteria of respective Escherichia coli, Klebsiella spp and Proteus spp and 100, 91 and 50 strains respectively were cultivated in a broth, and a dilution corresponding to 10^7 cfu/ml was prepared in Bertani trays. The test bacteria were then stamped on the new agar layer with the aid of a Steers Steel Pin Replicator (Steers E et al, J. Antibiot Chemother (1979,9,307). The plates were incubated at 37°C for twenty-four hours. Subsequent to incubation, the plates were read and compared with the reference plates. When reading the plates, "growth", "inhibition" or "zero growth" were registered for respective test organisms. The pH of all agar layers was measured, and those plates with a pH below 5.0 were re-tested with pH-adjusted agar; i.e. with agar to which a small quantity of glucose was added to counteract pH reduction. The percentile proportion of the total number of test organisms that had been inhibited or gave zero growth was calculated.

The diagram in Fig. 1 shows the percentage of the different strains of Escherichia coli, Klebsiella and Proteus that were inhibited by the presence of the selected strains of genera Lactobacillus and Lactococcus. It is evident that the Lactobacillus strains LB13 and LB16 have an inhibitory effect on practically all pathogenic strains. The Lactobacillus strain designated LB14 inhibited the growth of all E.coli strains, about 80% of the Proteus strains and slightly more than 30% of the Klebsiella strains. All of the Lactococci and Lactobacilli used had a growth inhibiting effect on some of the pathogenic strains. Of the Lactococci strains, inhibition of the highest percentage of pathogens was obtained with L26 and L9, both of which had an inhibitory effect on practically all E. coli strains and on a large percentage of the Klebsiella strains.

Example 2

The following tests were carried out with the intention of studying the transfer of lactic acid bacteria to the perineum of persons wearing panty liners. All test persons were females between 3-60 years of age. In some cases, the test was carried out between the menstruation periods of the test persons concerned. Test products were produced from conventional panty liners that included a liquid-permeable casing sheet, a liquid-impermeable back sheet, and an absorbent layer of chemical cellulose pulp, 100-200 g/m^2, sandwiched therebetween. A suspension of selected lactic acid bacteria was sprayed on the absorbent side of the test products, in a concentration of 10^9 cfu per product.

The presence of lactic acid bacteria in the perineum of the test persons was determined with the aid of a so-called swab test. In this case, bacteria were collected by dipping a sterile stick provided with a cotton-wool top into a sterile saline solution and stroking the top of the stick over a defined area of the skin. The presence of lactic acid bacteria in the perineum of twenty test persons was determined, measured, in this way, to obtain a so-called 0-sample (background sample). The test persons then wore the panty liner for five hours over a morning period. The panty liners were then removed and the presence of lactic acid bacteria again measured, immediately after removing the panty liners. This test was designated Sample 1. A further test, designated Sample 2, was run after a further four-five hours. The type of lactic acid bacteria was identified on each sampling occasion, in order to ascertain that none of the test persons was a natural carrier of the selected type of lactic acid bacteria added to the test products. The identification method used was API (API-system, La Balme les Grottes, 38390 Montalieu, Vercieu, France).

The results obtained with these measuring processes are defined in Table 1.

Table 1

		Presence of LAB* in the Perineum (cfu)		
TP	Flora	0-sample	Sample 1	Sample 2

1	Added LAB	0	0	0
	Own LAB	0	0	0
2	Added LAB	0	$4.0x10^1$	0
	Own LAB	0	0	0
3	Added LAB	0	$7.2x10^3$	$1.6x10^1$
	Own LAB	0	0	0
4	Added LAB	0	0	0
	Own LAB	0	0	0
5	Added LAB	0	$1.1x10^2$	$5.0x10^1$
	Own LAB	0	0	0
6	Added LAB	0	$5.0x10^1$	$3.0x10^1$
	Own LAB	+	+	0
7	Added LAB	0	$3.2x10^2$	$1.3x10^1$
	Own LAB	+	+	+
8	Added LAB	0	$2.7x10^3$	$6.0x10^1$
	Own LAB	0	0	0
9	Added LAB	0	$3.2x10^3$	$1.0x10^1$
	Own LAB	+	0	+
10	Added LAB	0	$7.6x10^2$	$7.0x10^1$
	Own LAB	+	+	+
11	Added LAB	0	$8.4x10^3$	$7.0x10^1$
	Own LAB	+	+	+
12	Added LAB	0	$3.5x10^3$	$8.0x10^1$
	Own LAB	0	0	0

13	Added LAB	0	$2.4x10^2$	$3.0x10^1$
	Own LAB	+	+	+
14	Added LAB	0	0	0
	Own LAB	+	+	+
15	Added LAB	0	$2.1x10^2$	$8.0x10^1$
	Own LAB	+	+	+
16	Added LAB	0	$1.6x10^4$	$4.0x10^1$
	Own LAB	0	0	0
17	Added LAB	0	$6.0x10^2$	$1.5x10^1$
	Own LAB	+	+	+
18	Added LAB	0	$9.3x10^2$	$1.0x10^1$
	Own LAB	0	0	0
19	Added LAB	0	$4.0x10^1$	$1.0x10^1$
	Own LAB	0	0	0
20	Added LAB	0	$7.2x10^3$	$7.0x10^1$
	Own LAB	0	0	0
Number of TP with added LAB		0	17	16

*LAB = lactic acid bacteria

0 = no lactic acid bacteria were found on the test persons

+ = presence of own LAB on the test persons

As evident from Table 1, the selected lactic acid bacteria added to the test products were activated when the test products were worn by the test persons, and that the selected

lactic acid bacteria were transferred to the perineum area of said persons and found to be present in said area even for a relatively long period of time after having removed the test products.

Example 3

A clinical study was carried out with the aim of illustrating the effect of adding lactic acid bacteria to the uro-perineal flora of children suffering from myelomeningocele. The study was blind, randomised and with cross-over design. Twenty-three children aged from two to seventeen participated in the study. All children were diaper wearers. Test products in the form of inserts with and without lactic acid bacteria were placed nearest the skin in the standard diaper.

The construction of the insert 1 used is shown in Fig. 2. Arranged uppermost on the insert 1, i.e. nearest the wearer's skin, is a liquid-permeable casing sheet 2 made of nonwoven material. A thin tissue layer 3 is disposed immediately adjacent to and inwardly of the casing sheet 2. The insert 1 also includes an absorbent pad 4 consisting of a mixture of cellulose fluff pulp and superabsorbent material. Placed on the surface of the absorbent pad 4 proximal to the tissue layer 3 is a layer of freeze-dried milk powder 5 with or without an admixture of selected, freeze-dried lactic acid bacteria in a concentration of 10^9 cfu per insert. The tissue layer 3, the absorbent pad 4 and the powdered milk 5 were enclosed between the liquid-permeable casing sheet 2 and a similar liquid-permeable backing sheet 6 of nonwoven material.

Two glue strings 7 were provided on the bottom surface 6 intended to lie proximal to the diaper in use, to enable the insert 1 to be fastened to the diapers worn by the test children. The glue strings 7 were protected by release paper 8, prior to placing the insert 1 in respective diapers.

The absorbent pad 4 was formed as an absorbent layer of 150 g/m^2 chemithermomechanical pulp admixed with 10% superabsorbent powder. The freeze-

dried milk powder, in the present case admixed with freeze-dried lactic acid bacteria, was sprinkled on the surface of the tissue layer 3 facing the absorbent pad 4. The tissue layer 3 had a weight per unit area of about 20 g/m^2 and was placed on top of the milk powder. The casing sheet 2 and the backing sheet 6 consisted of a nonwoven material having a weight per unit area of about 17 g/m^2 and were laminated in both sides by the absorbent unit 3-5. The thus produced insert 1 had an hourglass configuration with a largest width of about 9 cm, a smallest width of about 6.5 cm, and a length of about 24 cm.

The tests were continued over periods of 6 + 6 weeks with an intermediate period of three weeks. A urine culture was made prior to and during respective test periods (at two-week intervals) and a quantitative determination was made from the perineum and the urethra orifice. The number of bacteria obtained were transferred to a measurement scale between 0 and 5, where 0 denotes cfu <10^2, 1 denotes cfu between 10^2 and 10^3, and so on.

The results obtained are set forth in Table 2.

Table 2

		Insert without LAB*		Insert with LAB*		
Bacteria group	Local	M	SD	M	SD	Signi-cance
Potential	Perineum	1.93	0.84	1.52	0.89	Yes
Urinal	Urethra	1.62	1.12	1.29	0.95	No
Tract Pathogens	Urine	1.93	1.72	1.43	1.39	Yes

*LAB = lactic acid bacteria

M = mean value

SD = standard deviation

It is evident from Table 2 that the insert containing selected lactic acid bacteria provides a statistically significant ($p < 0.05$) reduction in the number of potential urinal tract pathogens (PUP) in the perineum and in the urine.

Example 4

The following tests were carried out with the intention of studying the survival of lactic acid bacteria in absorbent articles according to the invention. Test products were produced from conventional panty liners that included a liquid-permeable casing sheet, a liquid-impermeable back sheet, and an absorbent layer of chemical cellulose pulp, 100 - 200 g/m^2, sandwiched therebetween. About 150 µl of a suspension of selected lactic acid bacteria in saline skim milk sprayed on the absorbent side of the test products, in a concentration of about 9.0 x 10^7 cfu per product. Some test products were dried to a moisture content of less than 10 % (wt.) before they were packed in plastic packages, whereas other products were packed without any preceding drying step. The amount of living lactic acid bacteria was determined after 14, 28, 40 and 53 days, respectively using standard methods. The results of these tests are disclosed in Table 3 below:

Table 3

	dried panty liner		panty liner that has not been dried	
Day	cfu	cfu	cfu	cfu
0	9.0 x 10^7	7.0 x 10^7	9.0 x 10^7	7.0 x 10^7
14	7.5 x 10^6	6.1 x 10^6	3.6 x 10^5	8.0 x 10^5
28	7.2 x 10^7	2.6 x 10^7	2.1 x 10^4	1.0 x 10^3
40	8.9 x 10^7	3.2 x 10^7	1.4 x 10^4	1.0 x 10^3
53	7.0 x 10^7	3.0 x 10^7	1.1 x 10^4	1.0 x 10^3

As is evident from table 3 above, the amount of viable lactic acid bacteria does not decrease during the test period. However, the amount of viable lactic acid bacteria decreases considerably if no drying step is carried out before packaging.

The invention shall not be considered to be restricted to the aforedescribed examples, since it will be understood that a number of modifications and further embodiments are conceivable within the scope of the following Claims.

CLAIMS

1. An absorbent product, such as a diaper, sanitary napkin, panty liner, incontinence guard, an insert or like article, which contains lactic acid bacteria and is intended to be carried in contact with the user's skin in the perineum area, wherein lactic acid bacteria are arranged to be transferred to the user's skin and, when applicable, to the mucus membrane in the perineum area, to form a microbiological barrier that impairs the conditions for spreading and establishment of undesirable stains of microorganisms in said perineum area, **characterised** in that said absorbent product has been produced by applying a suspension of said lactic acid bacteria whereafter the absorbent product is dried to a moisture content of less than 10 %, preferably less than 5 % and most preferably less than 1 %, calculated as percentage of the weight of the absorbent core in the product.

2. A product according to claim 1, wherein the lactic acid bacteria exhibit an inhibiting effect against undesirable microorganisms in the user's perineum.

3. A product according to any one of the preceding Claims, wherein the lactic acid bacteria include one or more strains from the genera Lactobacillus, Lactococcus or Pediococcus.

4. A product according to Claim 3, wherein the lactic acid bacteria consist of one or more strains from the genus Lactobacillus.

5. A product according to any one of the preceding Claims, wherein the product includes a pH-lowering substance.

6. A product according to any one of the preceding Claims, wherein the product includes an auxiliary substance for facilitating survival of the lactic acid bacteria.

7. A product according to Claim 6, wherein said auxiliary substance is powdered skim milk.

8. An absorbent article according anyone of Claims 1-7, wherein lactic acid bacteria are disposed in or on a liquid-permeable casing sheet (2) included in said article.

9. An absorbent article according to anyone of claims 1-7, wherein lactic acid bacteria are located directly below the liquid-permeable casing sheet (2).

10. An absorbent article according to Claim 8 or 9, wherein lactic acid bacteria are disposed in or on an absorbent pad (4) included in said article.

11. An absorbent article according to any one of Claims 1-10, wherein the article contains lactic acid bacteria in numbers from between 10^4 and 10^{11} cfu, preferably between 10^6 and 10^{10} cfu.

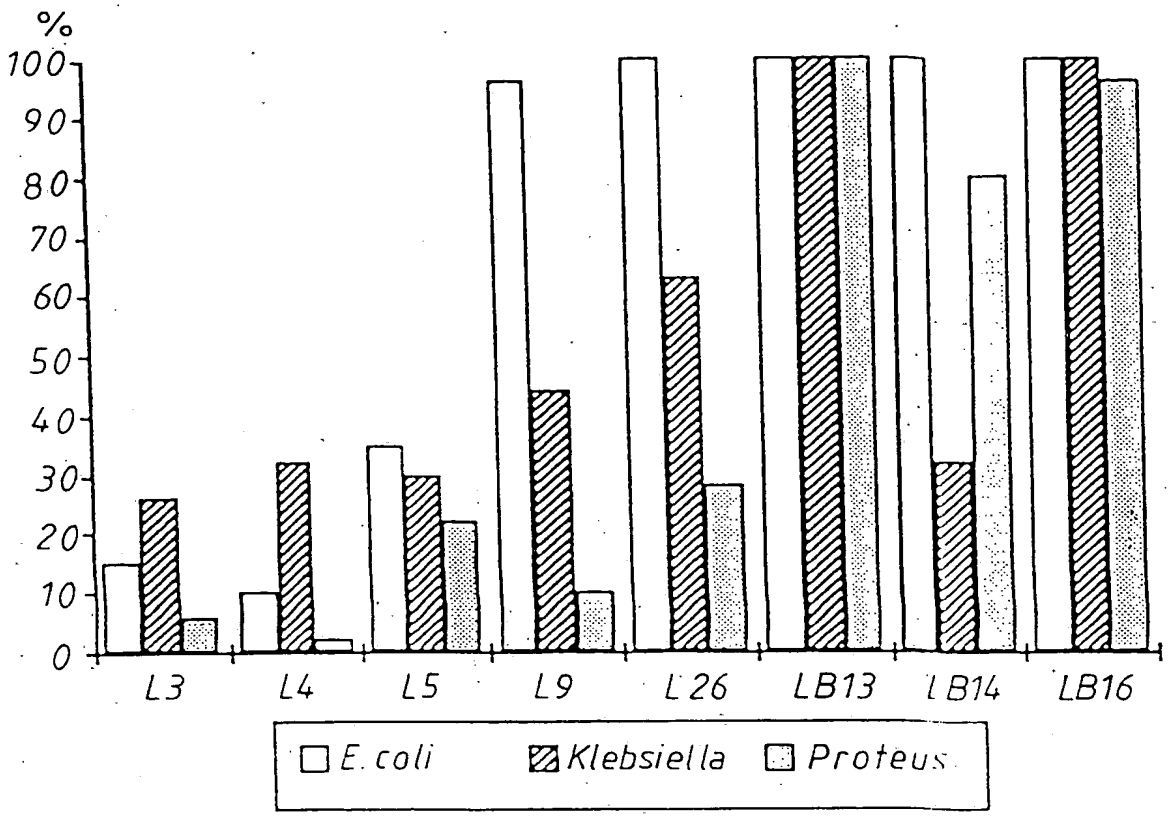

%
100
90
80
70
60
50
40
30
20
10
0
L3
L4
L5
L9
L 26
LB13
LB14
LB16
E. coli
Klebsiella
Proteus

FIG. 1

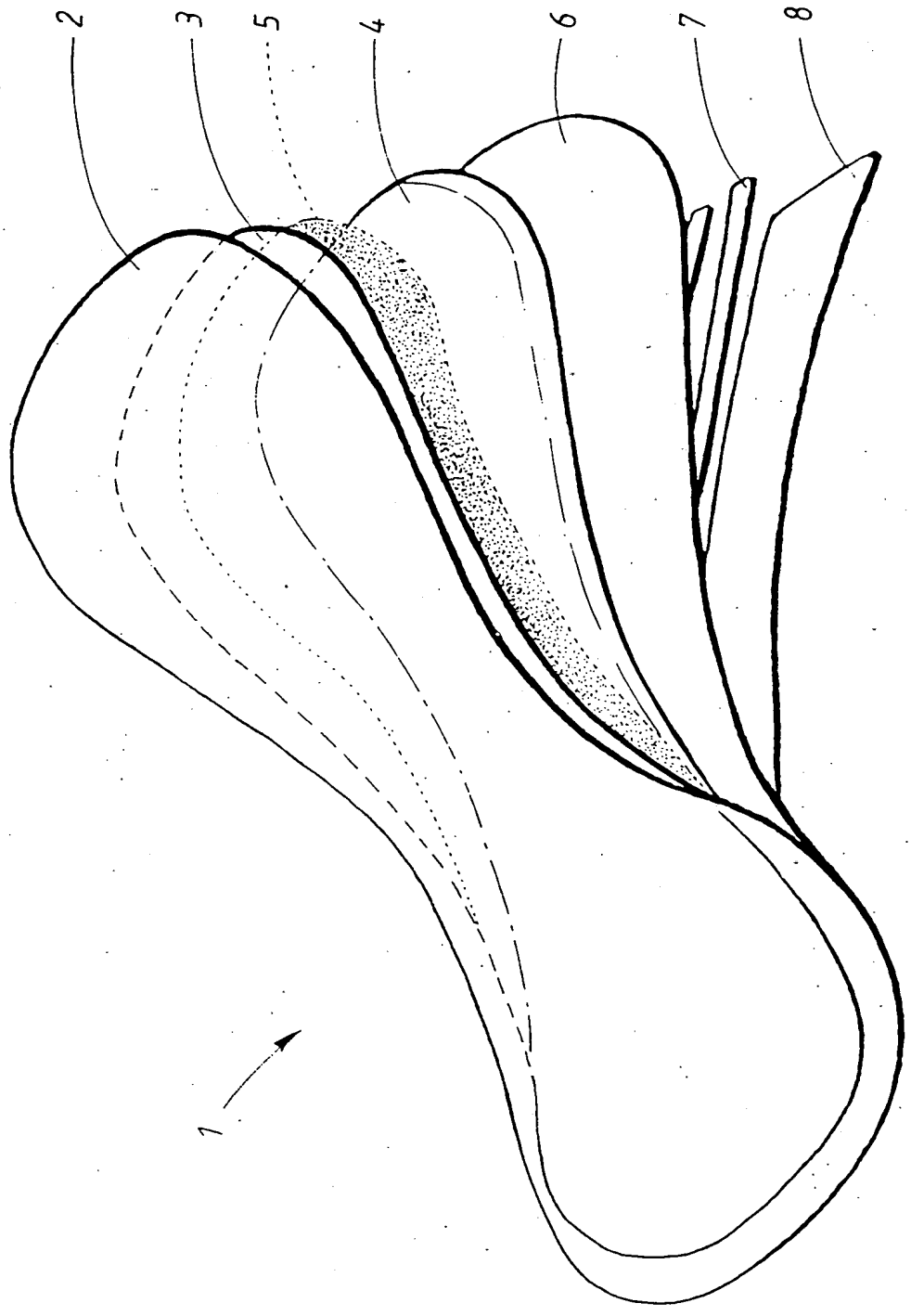


FIG.2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/01816

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61L 15/36, A61F 13/15
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61L, A61F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9213577 A1 (LECUR DEVELOPMENT IN SWEDEN AKTIEBOLAG), 20 August 1992 (20.08.92) --	1-11
A	WO 9309793 A1 (REID, GREGOR), 27 May 1993 (27.05.93) --	1-11
A	WO 9702846 A1 (SCA MÖLNLYCKE AB), 30 January 1997 (30.01.97) --	1-11

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 January 1999	29 January 1999 (29.01.99)
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. + 46 8 666 02 86	Authorized officer Jack Hedlund Telephone No. + 46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

P-1244

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61L 15/36, A61F 13/15

A1

(11) International Publication Number: **WO 97/02846**

(43) International Publication Date: 30 January 1997 (30.01.97)

(21) International Application Number: PCT/SE96/00891

(22) International Filing Date: 2 July 1996 (02.07.96)

(30) Priority Data:
9502588-8 13 July 1995 (13.07.95) SE

(71) Applicant *(for all designated States except US)*: SCA MÖLNLYCKE AB [SE/SE]; S-405 03 Gothenburg (SE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: RUNEMAN, Bo [SE/SE]; Jons väg 6, S-433 75 Jonsered (SE). ANDERSSON, Rolf [SE/SE]; Vallmovägen 11, S-435 31 Mölnlycke (SE). FORSGREN-BRUSK, Ulla [SE/SE]; Plommonvägen 35, S-435 43 Mölnlycke (SE). HOLM, Stig [SE/SE]; N Gimonäsvägen 25, S-907 38 Umeå (SE). GRAHN HÅKANSSON, Eva [SE/SE]; Nygatan 74, S-903 31 Umeå (SE).

(74) Agents: AXELL, Kristina et al.; H. Albihns Patentbyrå AB, P.O. Box 3137, S-103 62 Stockholm (SE).

(81) Designated States: AU, CA, CN, CZ, HU, IL, JP, MX, NO, NZ, PL, SK, TR, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: INHIBITING THE GROWTH OF BACTERIA IN ABSORBENT ARTICLES BY ADDING OTHER BACTERIA

(57) Abstract

The invention relates to absorbent articles, such as diapers and like articles and is concerned with methods for preventing undesirable odours and/or preventing the growth of undesirable microorganisms when the articles are in use, and also provides an absorbent article which can be worn for long periods of time without generating undesirable odours, incurring the risk of infection or having a negative effect on skin. Another object is to amplify the presence in the wearer's urogenital zone of microbiological flora that will assist in preventing the occurrence of urinal tract infections. These objects have been achieved by adding to the absorbent articles microorganisms which exhibit antagonistic properties against present undesirable strains of microorganisms, so as to restrain the growth of these or establishing of new undesirable species.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	**GB**	United Kingdom	**MW**	Malawi
AT	Austria	**GE**	Georgia	**MX**	Mexico
AU	Australia	**GN**	Guinea	**NE**	Niger
BB	Barbados	**GR**	Greece	**NL**	Netherlands
BE	Belgium	**HU**	Hungary	**NO**	Norway
BF	Burkina Faso	**IE**	Ireland	**NZ**	New Zealand
BG	Bulgaria	**IT**	Italy	**PL**	Poland
BJ	Benin	**JP**	Japan	**PT**	Portugal
BR	Brazil	**KE**	Kenya	**RO**	Romania
BY	Belarus	**KG**	Kyrgystan	**RU**	Russian Federation
CA	Canada	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SG**	Singapore
CH	Switzerland	**KZ**	Kazakhstan	**SI**	Slovenia
CI	Côte d'Ivoire	**LI**	Liechtenstein	**SK**	Slovakia
CM	Cameroon	**LK**	Sri Lanka	**SN**	Senegal
CN	China	**LR**	Liberia	**SZ**	Swaziland
CS	Czechoslovakia	**LT**	Lithuania	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**LV**	Latvia	**TJ**	Tajikistan
DK	Denmark	**MC**	Monaco	**TT**	Trinidad and Tobago
EE	Estonia	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**UG**	Uganda
FI	Finland	**ML**	Mali	**US**	United States of America
FR	France	**MN**	Mongolia	**UZ**	Uzbekistan
GA	Gabon	**MR**	Mauritania	**VN**	Viet Nam

INHIBITING THE GROWTH OF BACTERIA IN ABSORBENT ARTICLES BY ADDING OTHER BACTERIA

TECHNICAL FIELD

The present invention relates to absorbent articles such as diapers, incontinence guards, sanitary napkins and like articles, and is concerned with methods of preventing the generation of undesirable odours and/or the growth of undesirable microorganisms as the article is worn.

PROBLEMS

Many designs of absorbent articles of this kind are known to the art. Conventionally, the absorbent body of such articles is produced by dry-defibering cellulose pulp contained for instance in rolls, bales or sheets, and transforming the fluffed pulp to a pulp mat, sometimes while admixing so-called superabsorbents, which may be polymers capable of absorbing several times their own weight in water or body fluid.

The absorbent body may also include other components, for instance components that will improve the ability of the absorbent body to take-up and disperse liquid, and that will increase its coherency and its ability to resist deformation in use.

Articles of this kind are liable to generate undesirable odours when in use, caused among other things by microbial metabolism, biological or chemical decomposition of components in body fluids, such as urine or menstruation fluid, for instance.

Another problematic area associated with the use of absorbent articles is the risk of infection caused by pathogenic microorganisms.

Another problem is found in the working environmental risks that can arise when handling soiled diapers that contain a large quantity of microorganisms. The growth of microorganisms that can

take place in a soiled, used diaper during its storage after use can contribute to elevated odour problems and can also increase the risk of spreading undesirable microorganisms.

Another problem is the different forms of skin irritation and skin infections that can be caused directly or indirectly by microorganisms.

Microorganisms or their products that are known to contribute to the occurrence of undesirable odours, to cause infections in the urinal tract or to be associated with the occurrence of skin problems are, for instance such microorganisms as Proteus, Pseudomonas, Escherichia, Klebsiella, Enterococcus, Staphylococcus, Streptococcus and Candida.

DESCRIPTION OF THE KNOWN PRIOR ART

Different methods of alleviating the aforedescribed problems have been proposed. The International Patent Applications WO 91/11977 and WO 91/12031 describe methods of adsorbing undesirable odours in zeolite included in the absorbent article. U.S. Patent 4,385,632 describes the addition of copper salt to a diaper with the intention of preventing the decomposition of urea to ammonia and therewith prevent the occurrence of unpleasant smells.

U.S. Patent 3,794,034 describes the significance of pH in an absorbent article, and the significance of impregnating the article with a buffering substance by means of which the pH of the article can be maintained at between 3.5 and 6.0, which is advantageous from both the aspect of inhibiting the growth of undesirable bacteria, and therewith the generation of undesirable odours, and in avoiding a negative effect on the skin. European Patents EP 202127 and EP 311344 describe the adjustment of pH in absorbent articles that include superabsorbent material. Since buffering substances can have a negative effect on many superabsorbent materials, it is necessary to take separate measures to avoid undesirable effects. In the case of European Patent EP 202127, the superabsorbent material and buffering substances are placed in

mutually separated zones in the absorbent article. In the case of European Patent EP 311344, the buffering and the superabsorbent properties have been combined in one and the same material, and a separate bacteria-inhibiting substance has also been added.

The drawback with these described methods is that when only an odour-absorbing agent is added, the bacteria are still able to grow, and the bacteria inhibiting agents, which are often selective, can create risks, for instance, in the form of allergenic properties or negative ecological consequences when handling waste. Furthermore, the use of this type of agent involves the risk that resistant strains will occur.

It is known within the medicine and foodstuff technologies to use bioconservation with the aid of bacterial antagonism as a conserving method, and to inoculate special bacteria strains to favourize bacteria populations that are beneficial to the stomach and intestines, for health-promoting purposes. Examples in this respect are conventional yoghurt and soured milk, and also novel bioactive foodstuffs. This methodology also includes the use of bacteria such as so-called probiotic bacteria as a substitute for antibiotic bacteria.

Canadian Patent CA 1,298,556 teaches the medical use of selected strains of lactobacteria, wherein, among other things, whole cells or cell fragments of Lactobacillus are used to treat or preclude the occurrence of urinal tract infections. International Patent Application WO 93/09793 describes the use of lactobacteria and skimmed milk preparations for precluding or preventing urogenital infections. International Patent Application WO 92/13577 describes a tampon or sanitary napkin that has been impregnated with a culture of lactic-acid producing bacteria, preferably of the genus Pediococcus, isolated from healthy individuals. The tampon or sanitary napkin is intended for the prophylactic treatment of urogenital infections.

SUMMARY OF THE INVENTION

The object of the present invention is to provide an absorbent article of the kind mentioned in the introduction which will allow the article to be worn, even for a long period, without permitting microorganisms to grow or to become active to an extent such as to promote undesirable odours, to incur the risk of infection or to have a negative effect on the skin. Another object of the invention is to enable antagonistic microorganisms to be transferred to the wearer so as to amplify in the wearer's urogenital zone the occurrence of such microbiological flora as those that will assist in preventing the occurrence of urinary tract infection. These objects have been achieved in accordance with the invention by adding to the absorbent article microorganisms which exhibit antagonistic properties against present undesirable strains or arising undesirable strains of microorganisms present in the absorbent article or in the urogenital zone of the wearer during regular use of the absorbent article, wherein said microorganisms are added in an amount and have an activity such as to restrain the growth of undesirable species or establishing of new undesirable species of microorganisms.

BRIEF DESCRIPTION OF THE DRAWING

Fig. 1 illustrates in terms of percentage the proportion of different bacteria strains that exhibit inhibited growth in the presence of antagonistic strains.

DETAILED DESCRIPTION OF THE INVENTION

The object of the present invention is to provide an absorbent article of the kind mentioned in the introduction which will enable the article to be worn for a relatively long period of time without microorganisms being allowed to grow to an extent in which undesirable odours are generated, in which the risk of infection is created, or to an extent which will have a negative effect on skin. A further object of the invention is to enable antagonistic microorganisms to be transferred to the wearer so as to amplify in

the wearer's urogenital zone the presence of microbiological flora that will assist in preventing the occurrence of urinal tract infections.

Bacteria that cause unpleasant smells may belong to, for exemple, the family Enterobacteriaceae, e.g. Proteus mirábilis, Proteus vulgaris, Escherichia coli and Klebsiella.

Bacteria that cause urinal tract infections may belong to, for exemple, the families Enterobacteriaceae and Micrococcaceae or the genus Streptococcus. Exemples of species and genera are Escherichia coli, Proteus mirábilis, Enterococcus, Klebsiella, Staphylococcus and Streptococcus.

Microorganisms associated with skin infections are Ascomycetes, Pseudomonadaceae and Micrococcaceae and the genus Streptococcus, e.g. Candida albicans, Pseudomonas, Staphylococcus and Streptococcus.

The invention is based on microbiological antagonism. This implies that one microorganism or combinations of microorganisms inhibits/inhibit other microorganisms by competing for substrate, changing pH, forming enzymes, toxins, carbon dioxide, peroxides or antibiotics, so-called bacteriocines.

Antagonistic microorganisms may be naturally occurring microorganisms which are non-toxic and do not have any negative biological effect on humans, in the form of infections or skin changes.

Antagonistic microorganisms may also be produced biotechnically.

The addition to an absorbent article of microorganisms which exhibit antagonistic properties against such undesirable strains of microorganisms that are present when the absorbent article is worn regularly, is able to restrain the growth of these undesirable strains of microorganisms or establishing of new undesirable strains. Even some killing of microorganisms of undesirable species may occur. It is necessary for the microorganisms to have an

activity and to be added in amounts which will achieve the desired effect. Normally, this effect is achieved when the number of antagonistic microorganisms per absorbent article exceeds 10^6 cfu, preferably 10^8 cfu, more preferably 10^9 cfu. By regular use is meant in this case daily use of an article with the article replaced several times during a calendar day, as is the case with products intended for use with children or incontinent adults. The term regular use may also include the use relevant to sanitary napkins or tampons during a menstruation period.

One advantage afforded by the use of antagonistic microorganisms is that there is avoided an undesired selection pressure on the micro environment, such as favouring potential disease-promoting microorganisms and therewith the risk of developing pathogenic strains that are resistant to antibiotics and chemopharmaceutical preparations. Since the antimicrobial system is based on a natural, biological process, there is less risk of environmental ecological and toxic disturbances.

An antagonistic strain shall exhibit a growth-inhibiting effect on several of the aforesaid undesired microorganisms, with conventional interference techniques.

A desired antagonistic microorganism shall also be capable of surviving in storage and to retain its growth ability or its ability to retain its activity in the absorbent article when worn.

The microorganisms that exhibit antagonistic properties may be bacteria or other microorganisms, for instance fungi. When the antagonistic microorganisms are bacteria, these bacteria are preferably selected from the family Lactobacillaceae and particularly from the genera Lactobacillus or Lactococcus and preferably from the species Lactobacillus acidophilus, Lactobacillus curvatus, Lactobacillus plantarum or Lactococcus lactis.

An absorbent article produced in accordance with the invention may include a permeable outer sheet which is intended to lie proximal

to the wearer in use, a preferably liquid-impermeable backing sheet which is intended to lie distal from the wearer in use, and an absorbent structure placed between the outer sheet and the backing sheet. In some cases, an additional sheet in the form of, e.g., wadding or like material, may be placed between the outer sheet and the absorbent structure. The microorganisms exhibiting antagonistic properties may be placed in different parts of the absorbent article, for instance in the outer sheet, in the absorbent structure of the absorbent article, between two of the layers in the absorbent article, in a loose insert product in the absorbent article, or in some other way.

The following Examples illustrate the effect of antagonistic bacteria strains in more detail.

Example 1

Synthetic urine to which a microorganism growth medium had been added was used as a test liquid. The synthetic urine contained monoions, divalent ions, cations and anions and urea and was prepared in accordance with information contained in Geigy, Scientific Tables, Vol. 1, 8th ed., 1981, p. 53. The microorganism growth medium was based on information concerning Hook and FSA media for enterobacteria.

Example 2

Tests were carried out in accordance with the "agar overlay" method with the intention of studying bacterial antagonism. The method is based on the growth-inhibiting substance produced by the lactobacteria diffusing through an agar layer and inhibiting the growth of the test organisms.

Lactic acid bacteria, five strains of Lactobacillus and three strains of Lactococcus, were cultivated to an overnight culture in a suitable broth. Lactococcus were cultivated in M17 and Lactobacillus were cultivated in MRS. Agar (2%) of M17 and MRS (25 ml) respectively were mixed with 1.0 ml of respective bacteria and

moulded in a petri dish. The Agar plates were incubated overnight at 37°C. The plates that contained MRS were incubated in a CO_2 atmosphere. Reference plates were prepared in a corresponding manner, but without lactic acid bacteria. A further layer containing 25 ml agar was moulded on top of the layer present in the petri dishes and allowed to solidify.

The test organisms, in the form of Gram-negative bacteria of respectively Escherichia coli, Klebsiella spp and Proteus spp, and 100, 91 and 50 strains respectively, were cultivated in a broth and a dilution corresponding to 10^7 cfu/ml was prepared in a Bertani tray. The test bacteria were then stamped on the new agar layer with the aid of a so-called steers steel pin replicator. The plates were incubated at 37°C for twenty-four hours. At the end of the incubation period, the plates were scanned and compared with the reference plates. "Growth", "inhibition" and "zero growth" were registered for respective test organisms when scanning the plates. All agar layers were measured with regard to pH, and plates having a pH beneath 5.0 were retested with pH-adjusted agar.

The results are listed in Tables 1-6 and shown in Fig. 1. The total number of test organisms that had been inhibited or given zero growth were calculated in percentage units. The results show that the growth of respective test organisms Escherichia coli, Klebsiella and Proteus was greatly inhibited by the presence of genera Lactobacillus while some inhibition was obtained in the presence of genera Lactobacillus.

Test organism Escherichia coli

Lactococcus No.	Growth number	Inhibition number	Zero growth number	Inhibition or zero growth %
L 3	85	14	1	15
L 4	91	9	1	10
L 5	65	7	28	35
L 9	4	0	96	96
L 26	0	0	100	100

Table 1

Test organism Escherichia coli

Lactobacillus No.	Growth number	Inhibition number	Zero growth number	Inhibition or zero growth %
LB 13	0	0	100	100
LB 14	0	2	98	100
LB 16	0	2	98	100

Table 2

Test organism Klebsiella spp

Lactococcus No.	Growth number	Inhibition number	Zero growth number	Inhibition or zero growth %
L 3	68	16	7	25
L 4	71	23	6	32
L 5	64	10	17	29
L 9	51	10	30	44
L 26	34	36	21	63

Table 3

Test organism Klebsiella spp

Lactobacillus No.	Growth number	Inhibition number	Zero growth number	Inhibition or zero growth %
LB 13	0	0	91	100
LB 14	62	22	7	32
LB 16	0	0	91	100

Table 4

Test organism Proteus spp

Lactococcus No.	Growth number	Inhibition number	Zero growth number	Inhibition or zero growth %
L 3	47	2	1	6
L 4	49	1	0	2
L 5	39	7	4	22
L 9	45	4	1	10
L 26	36	8	6	28

Table 5

Test organism Proteus spp

Lactobacillus No.	Growth number	Inhibition number	Zero growth number	Inhibition or zero growth %
LB 13	0	0	50	100
LB 14	10	19	21	80
LB 16	2	7	41	96

Table 6

Example 3

Five test products were produced, each consisting of a pulp body that comprised a fibre mixture of 50% chemithermomechanical cellulose pulp and 50% chemical cellulose pulp with an addition of about 5% superabsorbent material. The pulp body was sandwiched between a permeable nonwoven material having a surface weight of 23 g/m^2 and an impervious backing sheet comprised of 33 µm polyethylene film. A layer of polyester wadding having a surface weight of 65 g/m^2 was placed between the nonwoven sheet and the pulp body. A mixture of freeze-dried lactobacteria of genus Lactobacillus was placed between the wadding and the pulp body in an amount corresponding to 10^8 bacteria per test product. 50 ml of synthetic urine according to Example 1 were then applied to the pulp body. The presence of lactobacteria in the groins and at the mouth of the urethra of five test subjects was measured, whereafter the subjects wore respective test products for two hours in the manner of a diaper or sanitary napkin. The presence of lactobac-

teria in the groins and at the mouth of the urethras of the test subjects was then again measured. The results are set forth in Table 7. It will be seen from the Table that the freeze-dried lactobacteria were activated when the test products were worn by the test subjects and that these lactobacteria were also transferred to the test subjects.

Test person	Start value urethra number lacto-bacteria	Start value groin number lacto-bacteria	After 2 hrs. urethra number lacto-bacteria	After 2 hrs. groin number lacto-bacteria
No. 1	No growth	No growth	$3.6x10^3$	$1.5x10^4$
No. 2	No growth	No growth	$2.8x10^3$	$2.7x10^2$
No. 3	No growth	No growth	$1.4x10^3$	$8.4x10^2$
No. 4	No growth	No growth	$3.8x10^6$	$4.0x10^3$
No. 5	No growth	No growth	$4.5x10^3$	$8.6x10^2$

Table 7

It will be understood that the invention is not restricted to the illustrated and described exemplifying embodiment thereof and that other embodiments are conceivable within the scope of the following Claims.

CLAIMS

1. An absorbent article, such as a diaper, a sanitary napkin, a tampon or the like to which microorganisms have been added, **characterized** in that the addition of microorganisms is selected so that when the absorbent article is worn regularly for a short or longer period, the microorganisms will exhibit antagonistic properties against present undesirable strains or arising undesirable strains of microorganisms present in the absorbent article or in the urogenital zone of the person wearing said article; and in that the microorganisms are added in such quantities and have such activity as to restrain the growth of undesirable species or establishing of new undesirable species of microorganisms.

2. An absorbent article according to Claim 1, **characterized** in that the microorganisms that exhibit antagonistic properties are bacteria.

3. An absorbent article according to Claim 2, **characterized** in that the antagonistic bacteria are taken from genera Lactobacillus or Lactococcus having antagonistic properties.

4. An absorbent article according to Claim 3, **characterized** in that the antagonistic bacteria are taken from species Lactobacillus acidophilus, Lactobacillus curvatus, Lactobacillus plantarum or Lactococcus lactis.

5. An absorbent article according to Claim 1, **characterized** in that the number of antagonistic microorganisms per absorbent article exceeds 10^6 cfu, preferably 10^8 and more preferably 10^9 cfu.

6. An absorbent article according to claim 1, **characterized** in that the microorganisms are such that they exhibit antagonistic properties against one or several undesirable strains from the families Enterobacteriaceae, Micrococcaceae, Pseudomonadaceae and Ascomycetes and the genus Streptococcus, preferably from the species Proteus mirábilis, Proteus vulgaris, Escherichia coli,

Candida albicans and the genera Klebsiella, Enterococcus, Staphylococcus, Streptococcus and Pseudomonas.

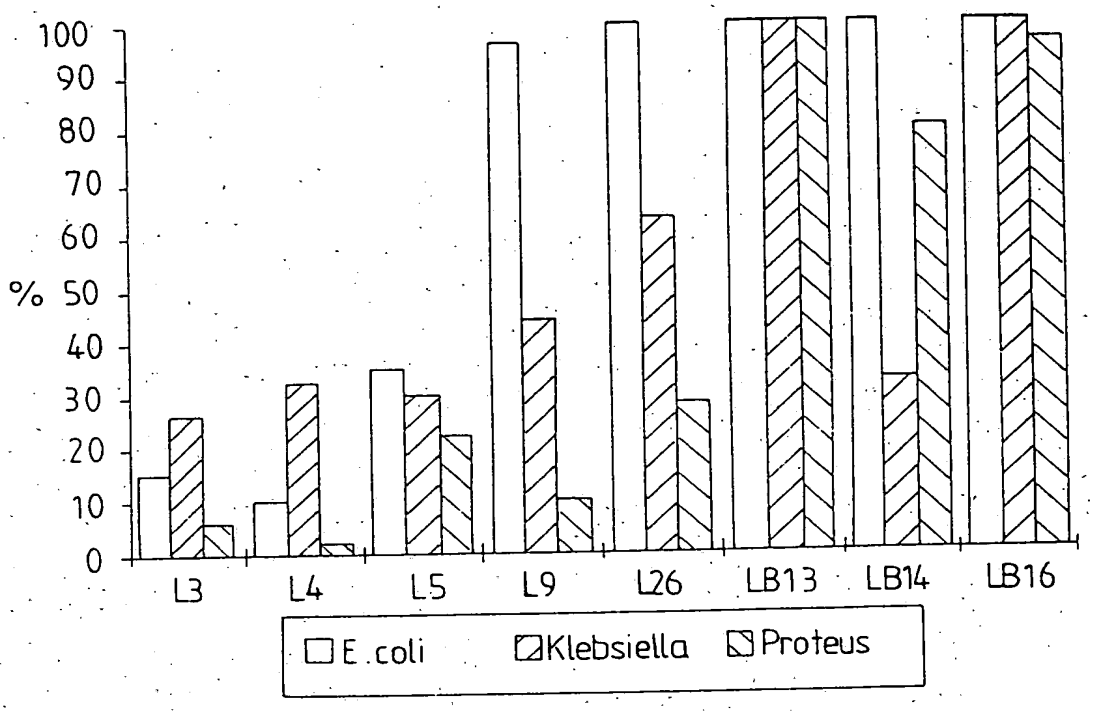
7. An absorbent article, such as a diaper, sanitary napkin, tampon or like article which includes a permeable outer material which is intended to lie proximal to the wearer in use, a preferably liquid-impermeable backing material which is intended to lie distal from the wearer in use, and an absorbent structure placed between the surface material and the backing material, wherein the absorbent article also includes microorganisms, **characterized** in that the microorganisms have been selected so that when the absorbent article is used regularly for a short or longer period, the microorganisms will exhibit antagonistic properties against present or arising undesirable strains of microorganisms present in the absorbent article or in the urogenital zone of the person wearing said article; and in that said microorganisms are added in such quantities and have such activity as to restrain the growth of undesirable species or establishing of new undesirable species of microorganisms.

8. An absorbent article according to Claim 7, **characterized** in that the microorganisms exhibiting antagonistic properties are placed in the surface material of the absorbent article.

9. An absorbent article according to Claim 7, **characterized** in that the microorganisms exhibiting antagonistic properties are placed in the absorbent structure of the absorbent article.

10. An absorbent article according to Claim 7, **characterized** in that the microorganisms exhibiting antagonistic properties are placed in a loose insert product in the absorbent article.

11. An absorbent article according to Claim 7, **characterized** in that the microorganisms exhibiting antagonistic properties are placed between two of the layers in the absorbent article.


100
90
80
70
60
% 50
40
30
20
10
0
L3
L4
L5
L9
L26
LB13
LB14
LB16
E.coli
Klebsiella
Proteus

INTERNATIONAL SEARCH REPORT

Information on patent family members

05/09/96

International application No.
PCT/SE 96/00891

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
SE-A- 8505491-4	21/05/87	NONE	
DE-A1- 2309575	05/09/74	NONE	